

CLAIMS

1. Method for synthesis of α -L-aspartyl-L-phenylalanine methyl ester by enzymatic deformylation of an N-formyl- α -L-aspartyl-L-phenylalanine compound, characterized in that N-formyl- α -L-aspartyl-L-phenylalanine or its methyl ester is treated with an enzyme having formylmethionyl peptide deformylase activity and having as a co-factor bivalent metal ions chosen from the group of group 5 to 11 metals from the periodic system of elements.
2. Method according to claim 1, characterized in that the enzyme having formylmethionyl peptide deformylase activity is an enzyme having the activity as described for EC 3.5.1.27.
3. Method according to any of claims 1 or 2, characterized in that the enzyme having formylmethionyl peptide deformylase activity contains the sequences of (i) HEXXXH, (ii) EGCLS and (iii) GXGXAAAXQ.
4. Method according to any of claims 1-3, characterized in that the enzyme having formylmethionyl peptide deformylase activity is obtainable from *E. coli*.
5. Method according to any of claims 1-4, characterized in that the bivalent metal ions are manganese, iron, cobalt and nickel ions.

6. Method according to claim 5, characterized in that the bivalent metal ions are iron and/or nickel ions.
7. Method according to claim 6, characterized in that the bivalent metal ions are iron ions and all treatments with the enzyme having formylmethionyl peptide deformylase activity are carried out in the presence of a stabilisation agent.
- 10 8. Method according to claim 7, characterized in that the stabilisation agent is catalase or a trialkylphosphine compound or derivative.
9. Method according to claim 8, characterized in that the stabilisation agent is catalase.
- 15 10. Method for the preparation and recovery of α -L-aspartyl-L-phenylalanine methyl ester by enzymatic deformylation of an N-formyl- α -L-aspartyl-L-phenylalanine compound, characterized in that either (i) a mixture of N-formyl- α - and N-formyl- β -L-aspartyl-L-phenylalanine or (ii) a mixture of N-formyl- α - and N-formyl- β -L-aspartyl-L-phenylalanine methyl ester is treated with an enzyme having formylmethionyl peptide deformylase activity and having as a co-factor bivalent metal ions chosen from the group of group 5 to 11 metals from the periodic system of elements, with the formation of α -L-aspartyl-L-phenylalanine or of its methyl ester, respectively, whereby in case α -L-aspartyl-L-phenylalanine is formed in the deformylation step a subsequent methylation

step of the phenylalanine carboxylic acid group is carried out, and the α -L-aspartyl-L-phenylalanine methyl ester is recovered.

11. Method according to claim 10, characterized in
5 that the enzyme having formylmethionyl peptide deformylase activity is an enzyme having the activity as described for EC 3.5.1.27.
12. Method according to any of claims 10 or 11, characterized in that the enzyme having
10 formylmethionyl peptide deformylase activity contains the sequences of (i) HEXXXH, (ii) EGCLS and (iii) GXGXAAXQ.
13. Method according to any of claims 10-12, characterized in that the enzyme having
15 formylmethionyl peptide deformylase activity is obtainable from *E. coli*.
14. Method according to any of claims 10-13, characterized in that the bivalent metal ions are manganese, iron, cobalt and nickel ions.
- 20 15. Method according to claim 14, characterized in that the bivalent metal ions are iron and/or nickel ions.
16. Method according to claim 15, characterized in
25 that the bivalent metal ions are iron ions and all the treatments with the enzyme having formylmethionyl peptide deformylase activity are carried out in the presence of a stabilisation agent.

17. Method according to claim 16, characterized in that the stabilisation agent is catalase or a trialkylphosphine compound or derivative.
18. Method according to claim 17, characterized in that the stabilisation agent is catalase.
19. Method for synthesis of α -L-aspartyl-L-phenylalanine methyl ester by enzymatic deformylation of an *N*-formyl- α -L-aspartyl-L-phenylalanine compound, characterized in that *N*-formyl-L-aspartic acid is coupled enzymatically, using thermolysin as the coupling enzyme, with L- or D,L-phenylalanine methyl ester, and that simultaneously, and in the same reaction vessel, the *N*-formyl- α -L-aspartyl-L-phenylalanine methyl ester formed by the coupling reaction is deformylated by an enzyme having formylmethionyl peptide deformylase activity and having as a co-factor bivalent metal ions chosen from the group of group 5 to 11 metals from the periodic system of elements and being present in the reaction system for the enzymatic coupling reaction.
20. Method according to claim 19, characterized in that, the α -APM so formed is recovered after the reaction has proceeded till a conversion of more than 40%.
21. Method according to any of claims 19 or 20, characterized in that the enzyme having formylmethionyl peptide deformylase activity is an enzyme having the activity as described for EC 3.5.1.27.

22. Method according to any of claims 19-21, characterized in that the enzyme having formylmethionyl peptide deformylase activity contains the sequences of (i) HEXXXH, (ii) EGCLS and (iii) GXGXAAQ.
- 5 23. Method according to any of claims 19-22, characterized in that the enzyme having formylmethionyl peptide deformylase activity has a deformylating activity towards (oligo)peptides with *N*-formylmethionine at their *N*-terminus, which is at least 10x higher, preferably at least 100x higher, and most preferred at least 200x higher than its deformylating activity towards *N*-formyl methionine.
- 10 15 24. Method according to any of claims 19-23, characterized in that the enzyme having formylmethionyl peptide deformylase activity is obtainable from *E. coli*.
- 20 25. Method according to any of claims 19-24, characterized in that the bivalent metal ions are manganese, iron, cobalt and nickel ions.
26. Method according to claim 25, characterized in that the bivalent metal ions are iron and/or nickel ions.
- 25 27. Method according to claim 26, characterized in that the bivalent metal ions are iron ions and all treatments with the enzyme having formylmethionyl peptide deformylase activity are carried out in the presence of a stabilisation agent.
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28. Method according to claim 27, characterized in
that the stabilisation agent is catalase or a
trialkylphosphine compound or derivative.

29. Method according to claim 28, characterized in
that the stabilisation agent is catalase.

5 30. Method for the synthesis of di- or oligopeptides
or derivatives thereof from two starting
materials, the first of which is an N-formyl
protected amino acid which is capable of
10 undergoing an enzymatic coupling reaction with a
second amino acid or derivative thereof, or with
a di- or oligo-peptide or derivative thereof,
thereby yielding an N-formyl protected reaction
15 compound, wherein the N-formyl protecting group
of the first starting material is retained during
the enzymatic coupling reaction with the second
starting material, whereby said protecting group
is cleaved off enzymatically, using an enzyme
20 having formylmethionyl peptide deformylase
activity and having as a co-factor bivalent metal
ions chosen from the group of group 5 to 11
metals from the periodic system of elements, from
the reaction compound at a substantially higher,
i.e. at least 10x higher, rate than from the
25 first starting material, and wherein two enzymes
are involved simultaneously for the enzymatic
coupling reaction between the starting materials
and the enzymatic deformylation of the reaction
compound.